

U.S. DEPARTMENT OF AGRICULTURE
GRAIN INSPECTION, PACKERS AND STOCKYARDS
ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
STOP 3630
WASHINGTON, D.C. 20090-3630

DON HANDBOOK
CHAPTER 6
9-11-06

CHAPTER 6

NEOGEN - VERATOX 5/5 DON TEST KIT

<u>Section Number</u>	<u>Section</u>	<u>Page Number</u>
6.1	TESTING AREA	6-1
6.2	EXTRACTION PROCEDURES.....	6-1
6.3	TEST PROCEDURES	6-2
6.4	REPORTING AND CERTIFYING TEST RESULTS	6-5
6.5	SUPPLEMENTAL ANALYSIS	6-6
6.6	CLEANING LABWARE	6-7
6.7	WASTE DISPOSAL	6-7
6.8	EQUIPMENT AND SUPPLIES	6-7
6.9	STORAGE CONDITIONS	6-8

6.1 TESTING AREA

The extraction solution and other materials used in the Veratox 5/5 test kit does not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

6.2 EXTRACTION PROCEDURES

- a. Standard Extraction Procedure - Testing Wheat, Oats, Barley, Malted Barley, Corn, and Rice at the 5 ppm Conformance Limit
- (1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker.
 - (2) Label the collection container with the sample identification.
 - (3) Thoroughly mix the ground sample and weigh a 50-gram portion.
 - (4) Place the ground 50-gram portion into an 18-ounce Nasco Whirlpack bag or similar type of sealable plastic bag.
 - (5) Add 250 ml of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.
 - (6) Let the extract sit for 2 minutes to enable some of the sample to settle before filtering the extract.
 - (7) Filter the extract by pouring through the filter paper into the labeled sample jar. Collect a minimum of 15 ml of the extract.

OR

Use a filtering syringe and push 1 - 2 ml of the extract through the syringe and collect the filtrate in a cuvette.

- (8) Dilute the sample extract 1:2 (1+1) with deionized or distilled water. (For example, add 1.0 ml of extract to 1.0 ml of deionized or distilled water).

- (9) Mix well.
- (10) Proceed to test analysis steps.

b. Optional Procedure - Testing Wheat, Oats, Barley, Malted Barley, Corn, and Rice at the 5 ppm Conformance Limit

- (1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker.
- (2) Label the collection container with the sample identification.
- (3) Thoroughly mix the ground sample and weigh a 50-gram portion.
- (4) Place the ground 50-gram portion into suitable size extraction container.
- (5) Add 500 ml of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.
- (6) Let material stand for 2 minutes to enable some of the sample to settle before filtering the extract.
- (7) Filter the extract by pouring through the filter paper into the labeled sample jar. Collect a minimum of 15 ml of the extract.

OR

Use a filtering syringe and push 1 - 2 ml of the extract through the syringe and collect the filtrate in a cuvette.

- (8) Proceed to the test analysis steps.

6.3 TEST PROCEDURES

a. Analysis Procedure.

- (1) Allow reagents, antibody coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test (approximately one hour).

- (2) Remove one red-marked mixing well for each sample to be tested, plus five red-marked wells to be used for controls. Place these wells in the microwell holder.

NOTE: The maximum number of test samples that can be run at one time is 19. Using two strips of 12 wells, designate 5 wells for the controls and the remainder of the wells for test samples.

- (3) Remove an equal number of antibody-coated wells. Immediately return antibody wells that will not be used to the foil pack with desiccant. Fold down ends of the pack and seal with tape to protect the antibody. Mark one end of the strip so that the wells can be identified after washing.
- (4) Mix each reagent by swirling the reagent bottle prior to use.
- (5) Using a new pipette tip for each, transfer 100 µl of conjugate from the blue-labeled bottle into each mixing well.
- (6) Using a new pipette tip for each, transfer 100 µl of control and sample extract into the mixing wells as shown below:

Well #	1	2	3	4	5	6	7	8	9	10	11	12
Sample	C 0	C 0.5	C 1.0	C 2.0	C 6.0	S1	S2	S3	S4	S5	S6	S7

Where C 0 is the zero control, C 0.5 is the 0.5 ppm control, C 1.0 is the 1.0 ppm control, C 2.0 is the 2.0 ppm control, and C 6.0 is the 6.0 ppm control. S1 is sample 1, S2 is sample 2, etc.

- (7) Using a 12- channel pipettor, mix the wells by pipetting the liquid up and down in the tips 3-4 times. Transfer 100 µl to the antibody wells and mix by sliding the microwell holder back and forth on a flat surface for 10 –20 seconds without splashing reagents from the wells. Incubate for **5 minutes** at room temperature (64 –86° F). Discard the red-mixing wells.

- (8) Dump the contents of the antibody wells. With a wash bottle or a running stream, fill each antibody well with deionized or distilled water and then dump the water out. Repeat this step 5 times, then turn the wells upside down and tap out on a paper towel until the remaining water has been removed.
- (9) Pour the needed volume of substrate from the green-labeled bottle into the green-labeled reagent boat, and with new tips on the 12-channel pipettor, prime and pipette 100 μ l of substrate into the wells and mix by sliding back and forth on a flat surface for 10-20 seconds. Incubate for **5 minutes**. Discard the remaining substrate and rinse the reagent boat with water.
- (10) Pour the Red Stop solution from the red-labeled bottle (same volume as prepared for substrate) into the red-label reagent boat. Eject the excess substrate from the 12-channel pipettor, prime the tips, and pipette 100 μ l of the Red Stop to each well. Mix by sliding back and forth on a flat surface. Discard the tips.
- (11) Wipe bottom of microwells with a dry cloth or towel and read in the Awareness Stat-Fax Model 321 PLUS Reader using a 650 nm filter. Air bubbles should be eliminated, as they could affect analytical results. Results should be read within 20 minutes of completion of the test.

b. Reading the Results with the Stat-Fax Model 321 PLUS Microwell Reader.

To begin from the "Ready" prompt, press Menu, key in test number 99, and then press Enter. The preprogrammed test menu will print. Use the test number listed for the DON 5/5 method. For example, if the DON 5/5 method is listed as menu number 5, press Menu, key in the test number 5, and then press Enter. Verify the calibration constants by running a strip. The constants should read:

C1	0.0
C2	0.5
C3	1.0
C4	2.0
C5	6.0

If no test for the DON 5/5 is present or the calibrators are not correct, contact Neogen Corporation.

- (1) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, then press enter. The carrier will advance into the reader, and it should start to print.

- (2) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N?"

Press "Yes" (1/A) to print the graph,

Press "No" (0) to skip this feature.

- (3) The screen will read, "Accept Curve Y/N ?"

Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended."

Press "No" (0) to end the test.

- (4) If a diluted sample extract (see Standard Extraction Procedure) is being analyzed, the reader value for the extract will need to be modified to adjust for the dilution of the extract. If the original extract was diluted 1+1 with water (this is an actual 1:2 dilution), the sample results are multiplied by 2. If the original extract was diluted 1+3 with water (this is an actual 1:4 dilution), the sample results are multiplied by 4.

NOTE: If the correlation coefficient is less than 0.98 or if the slope exceeds $-2.0 (\pm 0.5)$, the reader will print, "Invalid Calibration" and no results will be reported. If the slope value consistently reads outside these tolerances, contact Neogen as soon as possible to report these findings.

6.4 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

6.5 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** (5 ppm) is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON value at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 1 ml of the original extract (from 6.2 a (8), or 6.2 b (7), as applicable) with 2 ml of the extraction solution (distilled/deionized water). The total volume is 3 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\begin{array}{l} \text{True} \\ \text{DON} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result} \\ \text{Value} \end{array}$$

$$\begin{array}{l} \text{In this example:} \quad \text{True DON Value} = (15 \div 5) \times 3.1 \text{ ppm} \\ \quad \quad \quad = 3 \times 3.1 \text{ ppm} = 9.3 \text{ ppm} \end{array}$$

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limit of the test kit.

6.6 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

6.7 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

6.8 EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits:

- (1) 48 Monoclonal-Coated microwells.
- (2) 48 Red-Marked Mixing Wells.
- (3) 5 Yellow labeled Bottles - 1.5 ml each of 0, 0.5, 1.0, 2.0, and 6.0 ppm DON Controls.
- (4) 1 Blue-Labeled Bottle - DON-HRP Conjugate Solution.
- (5) 1 Green-Labeled Bottle - 24 ml K-Blue Substrate Solution.
- (6) 1 Red-Labeled Bottle - 32 ml Red Stop Solution.

b. Materials Required but not Provided:

- (1) Extraction Materials: Whirlpack Bags -18 oz., or equivalent. **NOTE: A larger size extraction container is required if using the 500 ml alternate procedure.**
- (2) Microwell Strip Reader: Awareness Technology STAT-FAX Model 321 PLUS Reader with a 650 nm filter.
- (3) 12-Channel multi-channel pipettor and pipette tips.
- (4) 100 µl pipettor and pipette tips.

- (5) 100 ml pipettor and pipette tips.
- (6) Deionized or Distilled Water.
- (7) Absorbent Materials: Paper Towels, Kay Dry paper or equivalent.
- (8) Waste receptacle.
- (9) Microwell holder.
- (10) Timer: 3-channel minimum.
- (11) Waterproof marker: Sharpie or equivalent.
- (12) Wash Bottle.
- (13) Reagent Boats.
- (14) Sample grinder.
- (15) Balance.
- (16) Neogen Filter Syringe.

6.9 STORAGE CONDITIONS

Test kits should be refrigerated at temperatures between 36° F and 46° F.